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13. ABSTRACT *(Maximum 200 words)*

Symbiotic associations are prevalent in all environments and are thought to be a driving force in evolution. These relationships encompass a spectrum of lifestyles, ranging from mutualistic to parasitic, and from extracellular to intracellular. This research was aimed at understanding the initiation, establishment and maintenance of cnidarian-algal associations. These associations are of global significance as corals and other related organisms form the foundation of coral reef ecosystems. These studies were among the first describe to examine the biochemical and molecular mechanisms underlying the establishment of the cnidarian-algal partnership. Further, the work described the natural life history of two associations, chosen for their amenability to laboratory manipulation and study.

We accomplished two major objectives:

Examination of changes in patterns of protein synthesis and gene expression that occur in host tissues during the initiation and establishment of symbiosis. Comparative techniques were be used to identify proteins and their encoding genes that are turned on by the onset of symbiosis. Using both comparative 2D protein profiles and subtracted libraries, we identified 4 symbiosis-enhanced genes that were expressed as a function of symbiosis.

Description of the role of algal infection in the early life histories of two cnidarians (hosts), a tropical coral Fungia scutaria *and a temperate anemone* Anthopleura elegantissima. We examined symbiosis onset in both species and also looked for the onset of symbiosis-specific gene expression.

14. SUBJECT TERMS

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Grant #: N00014-971-0101

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Institution: Department of Zoology, Oregon State University

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Award Period: 15 November 1996 - 30 September 1998 (23 months)

Objectives: Explore the molecular and genetic events governing the mutualistic endosymbiosis between cnidarians and dinoflagellates, a symbiosis of fundamental importance in marine ecosystems.

Approach: A. Identify and characterize symbiosis-specific genes and proteins. B. Compare transcript profiles between symbiotic and aposymbiotic animals. C. Examine changes in patterns of gene expression and protein synthesis during the onset of symbiosis.

Accomplishments

A. Characterization of symbiosis-specific genes and proteins:

We obtained peptide sequence of two symbiosis-specific proteins from *A. elegantissima* host tissue. From these sequences we designed degenerate PCR primers that we used to successfully amplify these sequences from symbiotic anemone cDNA using RT-PCR. We determined that one of these proteins is carbonic anhydrase (CA), an enzyme that has previously been described as being important in cnidarian-algal symbioses. This year we completed work on the CA sequence, documented its enhanced expression in symbiotic animals, and have published a paper in *Physiological Zoology*.

We have also amplified, cloned and sequenced another symbiosis-specific cDNA, p30, using the same technique that we used for CA. The deduced amino acid sequence has approximately a 30% homology, 58% similarity to a family of extrinsic membrane

proteins that function as adhesion and signaling molecules in a variety of organisms including mammals, *Drosophila*, sea urchins, green algae, and *Mycobacterium*. We have determined that p30 is present in other sybmiotic cnidarians, including a soft coral and a scleractinian coral. We have an almost complete manuscript to be submitted to *Comparative Physiology and Biochemistry*. We presented out data at the January 1999 meeting for the Society of Integrative and Comparative Biology

B. Construction a symbiosis-specific cDNA library using subtractive hybridization. Our original plan for identifying symbiosis-specific transcripts in *A. elegantissima* was to use the technique of differential display RT-PCR. My technician spent considerable time on this technique before determining that it was not the appropriate technique for our studies. Although we were able to generate many differential transcript profiles, we became mired, as many others have, in following up on false positives. We ultimately decided to focus our efforts on subtractive hybridizations instead. We are very pleased with the results. We have created a subtracted symbiosis-enhanced library that we have begun to screen using cDNA from symbiotic and non-symbiotic anemones. Our results thus far suggest that approximately one in five clones is symbiosis-specific.

By the end of the funding period we had successfully sequenced two symbiosis-enhanced genes. One shares significant homology, over 75%, with glyceraldehyde-3-phosphate dehydrogenase (GAPD) from other organisms. We confirmed that it is differentially expressed in symbiotic animals using RNA dot blots and semi-quantitative PCR, and Northerns. GAPD is a glycolytic pathway enzyme and is likely upregulated in the symbiotic state to handle algal translocation products from the host. The second symbiosis-enhanced sequence has high homology to a family of calmodulin-like proteins.

C. Examination of changes in patterns of gene expression and protein synthesis during the onset of symbiosis. We published a paper in *Biological Bulletin* documenting onset of symbiosis during larval development in the stony coral *Fungia scutaria*. We completed two field seasons of work in Hawaii on the onset of symbiosis in *F. scutaria*. We collected large quantities of symbiotic and non-symbiotic larvae that we transported back to OSU for analysis. We compared 2D protein

profiles of symbiotic and non-symbiotic larvae and found significant differences between the two. In the field, we also determined that both larvae and post-metamorphic polyps can acquire and maintain algae isolated from a variety of other cnidarian hosts. This has interesting implications for the level of specificity required between host and symbiont during symbiosis onset.

We also performed infection experiments on *A. elegantissima* larvae at OSU. *A. elegantissima* harbors both dinoflagellate and chlorophyte symbionts depending on environmental conditions and habitat. We successfully reared A. *elegantissima* larvae for one month in the laboratory and successfully infected them with dinoflagellate and chlorophyte symbionts, both separately and together. To our knowledge this is first time onset of symbiosis in *A. elegantissima* larvae has been studied.

Conclusions

This work on symbiosis-specific gene expression in cnidarian/algal symbioses was the first of its kind. Using comparative protein and comparative transcript techniques we successfully identified several genes that are significantly unregulated as a function of the symbiotic state. We also made considerable progress on understanding the events surrounding symbioses onset during the life history of cnidarian/algal associations. Future studies will elucidate the role of these 'symbiosis genes' in the symbiotic association.

Significance

Although cnidarian-algal associations are well studied at the macroscale level, relatively little is known about the complex biochemical, cellular and molecular mechanisms controlling the establishment and maintenance of theses associations. This study identified some of the genes and their proteins that are symbiosis-specific and determining how they function in the molecular interplay between partners.

Publications and Abstracts:

Weis, V. M. and W. S. Reynolds. In press. Carbonic anhydrase expression and synthesis in the sea anemone *Anthopleura elegantissima* are enhanced by the presence of dinoflagellate symbionts. *Physiol. Zool.* 72.

Schwarz, J. A., D. A. Krupp, and V. M. Weis. 1999. Late larval development and onset of symbiosis in the scleractinian coral *Fungia scutaria Biol. Bull. 196:70-79*

Weis, V. M. and W. S. Reynolds. 1998. Symbiosis-enhanced synthesis and expression of carbonic anhydrase in the sea anemone *Anthopleura elegantissima*. Am. *Zool. 38(5):94A*.

Schwarz, J. A., W. S. Reynolds, and V. M. Weis. 1998. The presence of symbiotic dinoflagellates in the sea anemone *Anthopleura elegantissima* enhances synthesis of a novel protein, p30, that is related to cell adhesion and signaling proteins in other organisms. *Am. Zool. 38(5):94A*.

Weis, V. M., J. von Kampen, and R. P. Levine. 1998. Techniques for exploring symbiosis-specific gene expression in cnidarian-algal associations. Pp 435 - 448 in: *Some Molecular Approaches to the Study of the Ocean,* K. E. Cooksey, Chapman Hall, London.